



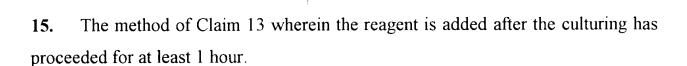
WHAT IS CLAIMED IS:

- 1. A method for determining the antigen specific activation of T cells from a human or animal subject comprising:
- (i) culturing a sample of whole blood of said human or animal with an antigen specific stimulus; and
- (ii) analyzing an aliquot of said cultured sample for expression of one or more intracellular cytokines and/or one or more early activation antigens in combination with one or more T cell markers, by means of multi-parameter flow cytometry.
- 2. The method of Claim 1 further comprising lysing the red blood cells and washing to remove the debris prior to the measuring step.
- 3. The method of Claim 1 further comprising providing exogenous costimulation of said cells by adding a costimulus to said sample contemporaneous with the addition of said antigen specific stimulus.
- 4. The method of Claim 3 wherein the costimulus is selected from the group consisting of ligands or antibodies to CD28, CD40, CD86, and CD118.
- 5. The method of Claim 1 wherein the antigen specific stimulus is selected from the group consisting of alloantigens, viral antigens, autoantigens, peptides, and proteins.





- 6. The method of Claim 5 wherein the antigen specific stimulus is selected from the group consisting of cytomegalovirus antigen, measles virus antigen, Mycobacterium tuberculosis antigen, and mumps virus antigen.
- 7. The method of Claim 1 wherein said intracellular cytokines are selected from the group consisting of γ -IFN, IL-2, IL-4, and TNF- α .
- 8. The method of Claim 1 wherein early activation antigen is CD69.
- 9. The method of Claim 1 wherein the sample is cultured with the antigen specific stimulus for a period of 24 hours or less.
- 10. The method of Claim 1 wherein the sample is cultured with the antigen specific stimulus for a period of 6 hours or less.
- 11. The method of Claim 1 further comprising culturing the sample with an immunosuppressive or immuno augmenting drug, simultaneous with the culturing with the antigen specific stimulus.
- 12. The method of Claim 1 wherein the sample of whole blood is obtained from a human or animal treated with an immunosuppressive or an immunoaugmenting drug.
- 13. The method of Claim 1 further comprising adding a reagent which blocks secretion of intracellular cytokines during the culturing of the sample with the antigen specific stimulus.
- 14. The method of Claim 13 wherein the reagent is Brefeldin A.



- 16. The method of Claim 1 further comprising adding a cationic chelating agent after the culturing is complete but prior to the measuring.
- 17. The method of Claim 16 wherein the cationic chelating agent is EDTA or EGTA.
- 18 The method of Claim 1 wherein the lysing of the red blood cells is accomplished using a lysing agent.

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